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Interactions of caffeine and nitric oxide on pentylenetetrazole-induced clonic and tonic seizures in mice

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Abstract

Background: Caffeine, a non-selective antagonist of adenosine receptors (ARs), may affect the anticonvulsant properties of adenosine through modulation of the nitric oxide (NO) pathway.

Objectives: This study aimed to investigate the impact of caffeine administration on pentylenetetrazole (PTZ)-induced seizure threshold and its potential interaction with the NO pathway.

Methods: Male NMRI mice (25-30 g, 5-6 weeks old) were administered varying doses of caffeine (10, 50, and 100 mg/kg), L-arginine (50, 100, and 500 mg/kg) as a substrate for nitric oxide synthase (NOS), sodium nitroprusside (SNP, 3, 6, and 9 mg/kg) as a NO donor, or N(G)- nitro- l- arginine methyl- ester (L- NAME, 5, 15, and 30 mg/kg) as a non-selective NOS inhibitor prior to PTZ infusion. In pre-treatment groups, sub-effective doses of L-arginine (50 mg/kg), SNP (3 mg/kg), or L-NAME (5 mg/kg) were administered before caffeine at doses of 10 or 100 mg/kg. The threshold for the onset of myoclonic twitch (MC) or tonic hind limb extension (THE) was evaluated following PTZ infusion.

Results: A dose of 100 mg/kg caffeine did not significantly decrease the threshold for the onset of MC twitch. However, all other doses of caffeine significantly reduced the threshold for the onset of MC twitch ($P<0.01$) or THE ($P<0.01$). Pre-treatment with L-arginine or SNP exhibited a more proconvulsant effect, whereas pre-treatment with L-NAME notably mitigated the proconvulsant effect of caffeine.

Conclusion: The study findings indicate that the effects of caffeine on seizure thresholds vary depending on the dose and seizure stage. Moreover, the impact of caffeine on seizure thresholds may involve an interaction with the NO-cyclic guanosine monophosphate (cGMP) pathway.

Keywords: Seizure, Caffeine, Nitric oxide, N(G)-nitro-l-arginine methyl-ester (L-NAME), L-arginine, Pentylenetetrazole.

Introduction

Caffeine (1,3,7-trimethylxanthine), a purine alkaloid, is one of the most widely consumed pharmacologically active substances globally. Common sources of caffeine include coffee, soft drinks, chocolate, and tea.[1] Structurally similar to adenosine, caffeine acts as a mixed competitive antagonist with nearly equal affinity for adenosine A1 receptor (A_1R) and adenosine A_2A receptor ($A_{2A}R$).[2,3]

In terms of the central nervous system (CNS), caffeine exhibits dose-dependent stimulatory effects, leading to behavioral arousal, increased alertness, hyper-excitability, restlessness, and tremors.[4] Studies in both animals and humans have shown the proconvulsant effects of caffeine. High doses of caffeine (200 mg/kg and above) have been found to induce seizures in rodents and primates.[5] A recent systematic review also suggests that caffeine may increase seizure susceptibility.[6] However, the relationship between caffeine, seizures, and epilepsy remains a contentious topic. The dose-dependent effects of caffeine on seizures have been questioned in certain animal models of epilepsy.[7-9] Additionally, some clinical studies have reported that daily consumption of high-dose caffeine (up to 400 mg) does not necessarily increase seizure risk.[10,11] These conflicting findings may stem from variations in caffeine dosage or animal models used. Our previous study

focused solely on the time to onset of clonic seizure threshold,[9] while tonic-clonic seizures are induced in specific animal models of epilepsy such as maximal electroshock (MES).[7] Therefore, reporting both clonic and tonic seizures may help clarify these discrepancies.

Nitric oxide (NO) is a well-known gaseous signaling molecule produced from L-arginine by various nitric oxide synthase (NOS) isoforms, including endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS).[12] NO plays a role in regulating behavioral, cognitive, and emotional processes such as anxiety, depression, learning, and seizure activity.[13] Its effects on seizures can be either anticonvulsant or proconvulsant, depending on the seizure model or administration route.[14-16] Adenosine influences NO production by activating A1R and A_2AR.[17] Given that caffeine antagonizes adenosine receptors (ARs), it can potentially modulate NO production. Notably, the non-specific NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) has been shown to counteract the effects of caffeine on antinociception and locomotor activity.[18,19] Our previous research indicated that 100 mg/kg caffeine reduced NO metabolite levels,[9] while caffeine also counteracted ethanol-induced cerebral artery constriction through endothelial NO.[20]

Objectives

It appears that caffeine's mechanism of action extends beyond AR antagonism. Other signaling pathways, such as the NO-cyclic guanosine monophosphate (cGMP) pathway, likely play a role in the central effects of caffeine. Therefore, this study aims to explore the impact of acute caffeine administration on pentylenetetrazole (PTZ)-induced clonic and tonic seizure thresholds in mice. Additionally, we will investigate the potential interaction between caffeine and NO in modulating seizure susceptibility.

Methods

Animals

NMRI male mice weighing 25-30 g (age 5–6 weeks), bred in the animal house of the Physiology Research Center at Kashan University of Medical Sciences, were utilized in this study. The animals were housed in standard polypropylene cages at a constant temperature (22±2 ºC) and humidity (50-55%) with a 12-hour light and 12-hour dark cycle.

Chemicals

The chemicals and drugs used in this study included PTZ, caffeine, L-arginine (a substrate for NOS), sodium nitroprusside (SNP) (a NO donor), and L-NAME. All the mentioned drugs were obtained from Sigma (USA). PTZ (0.5% solution) was prepared in heparinized sterile saline 0.9% and administered as an intravenous (iv) infusion. All drugs were dissolved in normal saline solution at the desired concentrations and administered via the intraperitoneal (ip) route in a volume of 5 ml/kg of body weight.

Experiments

The animals were randomly divided into 19 groups, with each experimental group consisting of 8 mice based on pilot experiments and previous studies on this seizure model.[9,21]

In experiment 1 (comprising 4 groups), animals received an ip injection of different doses of caffeine (10, 50, and 100 mg/kg) 30 minutes before PTZ infusion. Control animals received the same volume of saline. Based on this experiment, caffeine doses of 10 and 100 mg/kg were used in subsequent experiments to study possible interactions with the NO-cGMP pathway.

In experiment 2 (comprising 9 groups), mice were acutely administered different doses of L-arginine (50, 100, and 500 mg/kg), SNP (3, 6, and 9 mg/kg), or L-NAME (5, 15, and 30 mg/kg) 45 minutes before PTZ infusion.

In experiment 3 (comprising 6 groups), we examined the effect of pre-treatment with non-effective doses of L-arginine (50 mg/kg), SNP (3 mg/kg), or L-NAME (5 mg/kg) 15 minutes before administration of 10 or 100 mg/kg caffeine. In all pre-treatment groups, PTZ was infused 30 minutes after caffeine administration.

Seizure Induction

PTZ concentration (5 mg/ml) was dissolved in heparinized sterile saline (0.9%) to prepare a fresh solution for intravenous (iv) infusion. The dose and infusion rate of PTZ were 5 mg/ml in saline and 0.5 ml/min, respectively. Before testing, each mouse was weighed, restrained in a clear acrylic plastic cage, and its tail was immersed in a warm water bath (40°C) for 1 minute to dilate the tail veins. The lateral tail vein of the mouse was catheterized with a 30-gauge dental needle attached to No.10 polyethylene tubing, secured to the tail by adhesive tape. The PE tubing (approximately 50 cm in length) was connected to a 10 ml plastic syringe containing the PTZ solution mounted into a syringe pump (Top, Japan). The PTZ solution was then infused into the tail vein of the freely moving mouse at a constant rate of 0.5 ml/min. The times (in seconds) from the start of infusion to the

appearance of myoclonic twitch (MC twitch) or tonic hind limb extension (THE) were recorded for each mouse. The recorded times were then converted to mg/kg PTZ for each mouse based on the PTZ dose administered and time-related factors.[9, 21-22]

Statistical analysis

All data were presented as mean ± standard error (SE). The significance of differences in seizure threshold was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. All statistical analyses were performed with SPSS (version 24.0, SPSS Inc, Chicago, IL, USA). A "P-value" less than 0.05 was considered significant.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. All experiments were conducted following the guidelines for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985) and were approved by the Research and Ethics Committee of Kashan University of Medical Sciences (ethics code: IR.KAUMS.MEDNT.REC.1398.075), Kashan, Iran.

Results

Effect of caffeine on the threshold dose for the onset of MC twitch and THE

One-way ANOVA for analyzing the threshold dose for the onset of MC twitch showed a significant difference among the groups administered with caffeine at doses of 10, 50, and 100 mg/kg [Figure 1A]. Post hoc analysis revealed that caffeine at doses of 10 and 50 mg/kg significantly decreased the threshold dose for the onset of MC twitch compared to saline-treated control animals ($p<0.01$ and $P<0.05$, respectively).

One-way ANOVA for analyzing the threshold dose for the onset of THE also showed a significant difference ($F_{3,28}=27$, $P<0.001$) among the groups administered with caffeine at doses of 10, 50, and 100 mg/kg [Figure 1B]. Post hoc comparisons indicated a significant proconvulsant effect for all doses of caffeine (10, 50, and 100 mg/kg) compared to saline-treated control animals ($P<0.05$, $P<0.001$, and $P<0.001$, respectively). The lowest and highest doses of caffeine (10 and 100 mg/kg, respectively) were selected for further experiments to explore the potential interaction of caffeine with the NO-cGMP pathway.

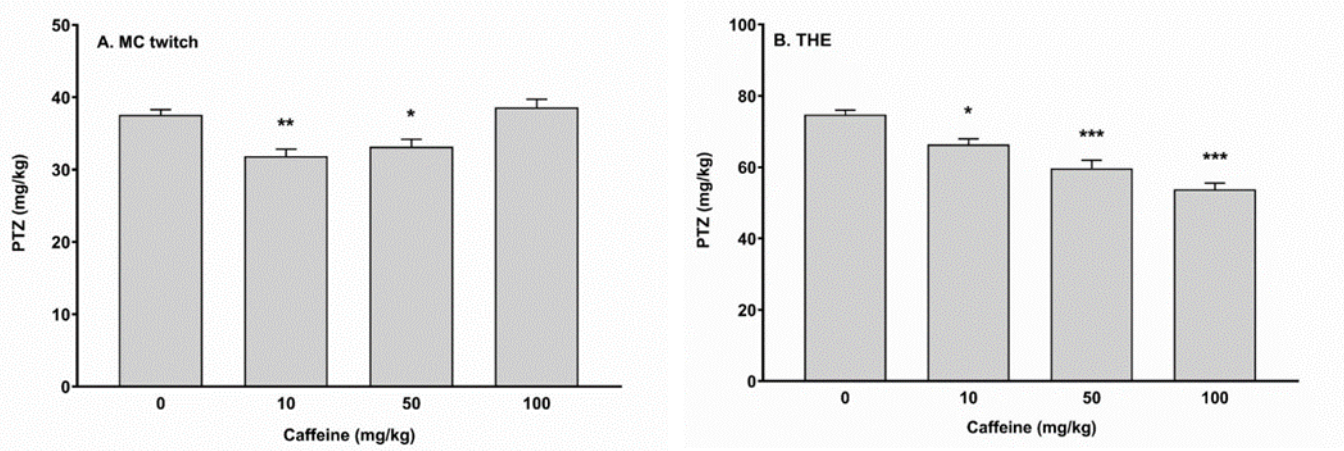

Figure 1. Effect of acute administration of caffeine (10, 50, and 100 mg/kg, *ip*) on the PTZ-induced seizure threshold. Mice were infused with PTZ and observed for the onset to MC twitch (A) and THE (B). *P<0.05, **P<0.01 and ***P<0.001 in comparison with saline-treated control group (mean ± S.E.M, n=8).

Effect of per se doses of L-Arginine, SNP, or L-NAME on the threshold dose for the onset of MC twitch and THE

When L-arginine (10, 100, and 500 mg/kg), SNP (3, 6, and 9 mg/kg), or L-NAME (5, 15, and 30 mg/kg) was administered per se, one-way ANOVA for analyzing the threshold dose for the onset of MC twitch showed a significant effect for L-arginine ($F_{3,28}=5.08$, $p=0.006$) and SNP ($F_{3,28}=5.32$, $p=0.005$), but not for L-NAME ($F_{3,28}=0.89$, $p=0.46$) [Figure 2A]. Post hoc comparisons indicated a significant proconvulsant effect for L-arginine at doses of 100 and 500 mg/kg ($P<0.05$ and $P<0.01$, respectively) or SNP at a dose of 9 mg/kg ($P<0.05$).

When L-arginine (10, 100, and 500 mg/kg), SNP (3, 6, and 9 mg/kg), or L-NAME (5, 15, and 30 mg/kg) was administered per se, one-way ANOVA for analyzing the threshold dose for the onset of THE showed a significant effect for SNP ($F_{3,28}=4.05$, $p=0.016$), but not for L-NAME ($F_{3,28}=1.58$, $p=0.22$) or L-arginine (($F_{3,28}=1.96$, $p=0.14$) [Figure 2B].

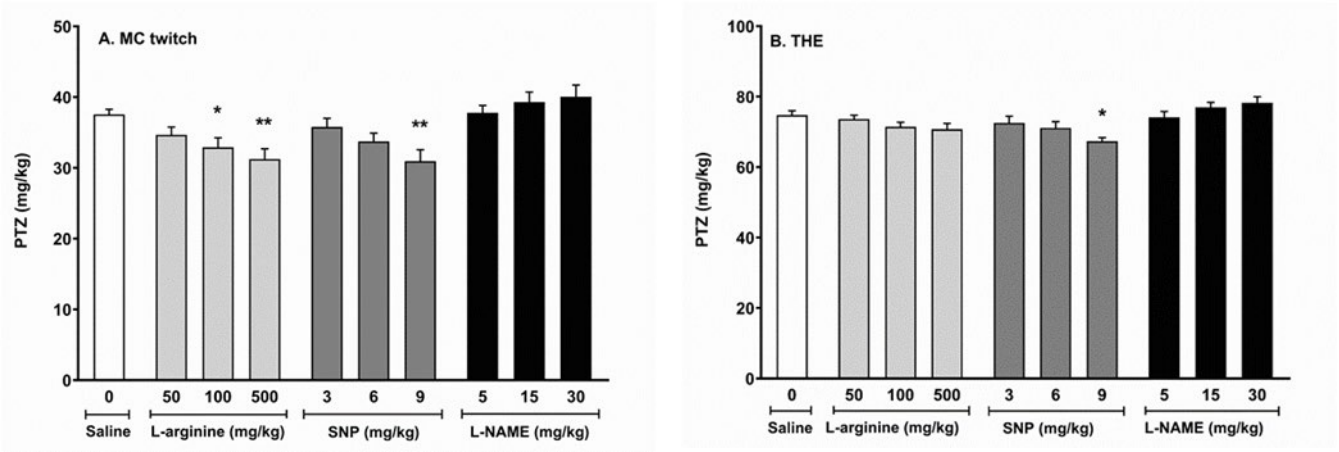

Figure 2. Effects of pretreatment with L-arginine (50, 100, and 500 mg/kg), SNP (3, 6, and 9 mg/kg) or L-NAME (5, 15, and 30 mg/kg) on the PTZ-induced seizure threshold. Mice were infused with PTZ and observed for the onset to MC twitch (A) and THE (B). *P<0.05 and **P<0.01 in comparison with the saline-treated control group (mean ± S.E.M, n=8).

Effect of pretreatment with L-Arginine, SNP, or L-NAME before caffeine (10 mg/kg) on the threshold dose for the onset of MC twitch and THE

One-way ANOVA revealed a significant effect on the onset of MC twitch ($F_{4,35}$=23, p<0.001) when non-effective doses of L-arginine (50 mg/kg), SNP (3 mg/kg), or L-NAME (5 mg/kg) were administered before caffeine (10 mg/kg) [Figure 3A]. Post hoc analysis indicated that L-arginine or SNP significantly decreased (p<0.05 and p<0.01, respectively), while L-NAME significantly increased (p<0.05) the threshold dose for the onset of MC twitch compared to the saline+caffeine 10 mg/kg group.

One-way ANOVA also showed a significant effect on the onset of THE ($F_{4,35}$=16.3, p<0.001) when non-effective doses of L-arginine (50 mg/kg), SNP (3 mg/kg), or L-NAME (5 mg/kg) were administered before caffeine (10 mg/kg) [Figure 3B]. Post hoc analysis revealed that L-NAME significantly increased the threshold for the onset of MC twitch compared to the saline+caffeine 10 mg/kg group.

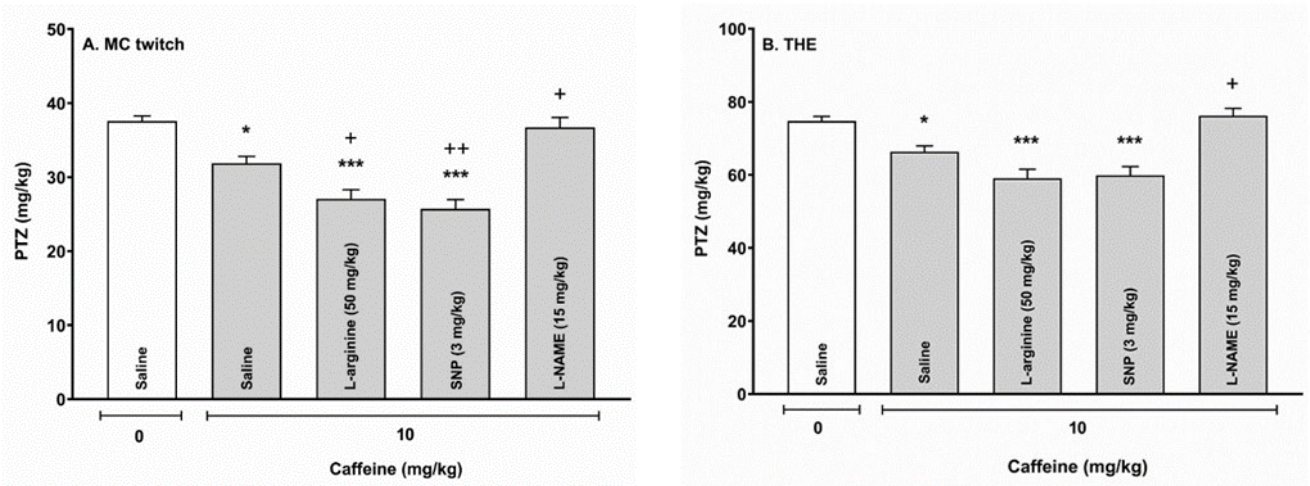

Figure 3. Effects of pre-treatment with L-arginine, SNP or L-NAME before caffeine (10 mg/kg) in mice. L-arginine (50 mg/kg), SNP (3 mg/kg), L-NAME (5 mg/kg) or saline were injected 15 min before a proconvulsant dose of caffeine (10 mg/kg). Mice were infused with PTZ 30 min alter and observed for the onset to MC twitch (A) and THE (B). *P<0.05, and ***P<0.001 in comparison with saline-treated control group, +P<0.05 and ++P<0.01 in comparison with saline+caffeine 10 mg/kg group (mean ± S.E.M, n=8).

Effects of pretreatment with L-Arginine, SNP, or L-NAME before caffeine (100 mg/kg) on the threshold dose for the onset of MC twitch and THE

One-way ANOVA demonstrated a significant effect on the onset of MC twitch (F4,30=9.39, p<0.001) when non-effective doses of L-arginine (50 mg/kg), SNP (3 mg/kg), or L-NAME (30 mg/kg) were administered before caffeine at a dose of 100 mg/kg [Figure 4A]. Post hoc analysis showed that L-arginine and SNP, but not L-NAME, further decreased the threshold dose for the onset of MC

twitch compared to the saline+caffeine 100 mg/kg group ($p<0.01$ and $p<0.001$, respectively).

Similarly, one-way ANOVA revealed a significant effect on the onset of THE ($F_{4,30}=49.1$, $p<0.001$) when non-effective doses of L-arginine (50 mg/kg), SNP (3 mg/kg), or L-NAME (30 mg/kg) were administered before caffeine at a dose of 100 mg/kg [Figure 4B]. Post hoc analysis indicated that L-arginine and SNP significantly decreased ($p<0.01$ and $p<0.001$, respectively), while L-NAME significantly increased ($P<0.001$) the threshold dose for the onset of THE compared to the saline+caffeine 100 mg/kg group. Notably, pretreatment with L-NAME abolished the proconvulsant effect of 100 mg/kg caffeine.

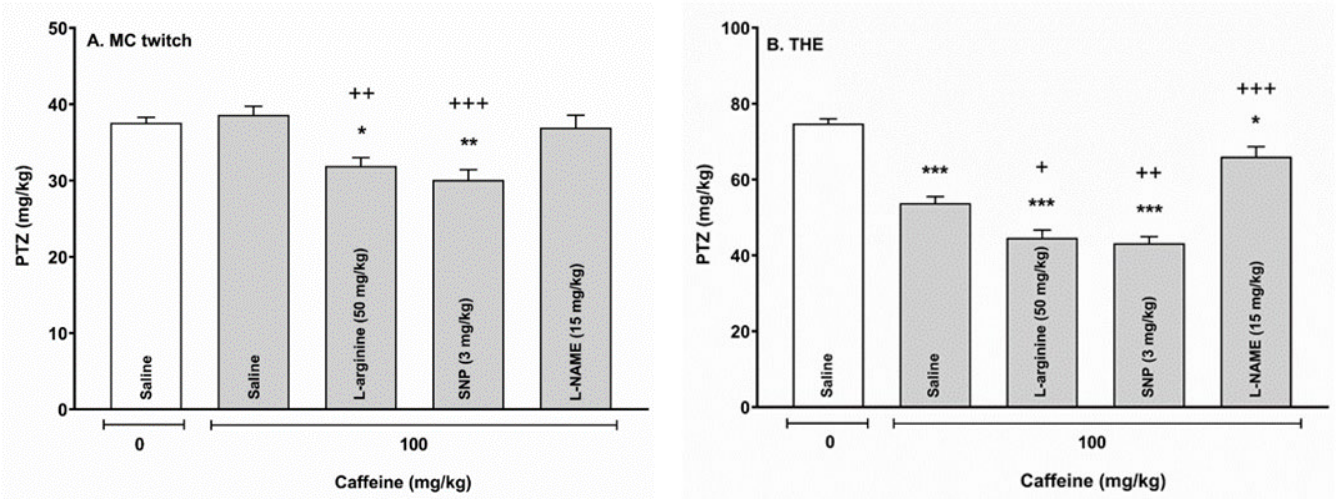

Figure 4. Effects of pre-treatment with L-arginine, SNP or L-NAME before caffeine (100 mg/kg) in mice. L-arginine (50 mg/kg), SNP (3 mg/kg), L-NAME (15 mg/kg) or saline were injected 15 min before a proconvulsant dose of caffeine (100 mg/kg). Mice were infused with PTZ 30 min alter and observed for the onset to MC twitch (A) and THE (B). *P<0.05, **P<0.01 and ***P<0.001 in comparison with saline-treated control group, $^{+}$P<0.05, $^{++}$P<0.01, and $^{+++}$P<0.001 in comparison with saline+caffeine 100 mg/kg (mean ± S.E.M, n=8).

Discussion

PTZ binds to a distinct site separate from the picrotoxin binding site on the GABA receptors.[23] PTZ-induced GABA inhibition enhances excitatory transmission in the forebrain (the origin of MC twitch) and hindbrain (the origin of THE) structures.[22] The intravenous PTZ seizure threshold test serves as an extremely sensitive model for evaluating seizure thresholds. This model assesses the threshold for both MC twitch and THE, enabling a separate examination of drug effects on different seizure types in the same animals.

Consistent with our previous study,[9] caffeine at doses of 10 and 50 mg/kg exhibited a proconvulsant effect and significantly reduced the threshold dose for the onset of MC twitch and THE. Conversely, at a dose of 100 mg/kg, caffeine notably decreased the threshold dose for the onset of THE without affecting MC twitch [Figure 1]. We also investigated the potential involvement of the NO–cGMP pathway in caffeine's effects (at 10 and 100 mg/kg). Our findings demonstrated interactions between L-arginine (an NOS precursor), SNP (a NO donor), or L-NAME (a non-selective NOS inhibitor) with caffeine [Figure 4].

Caffeine (1,3,7-trimethylxanthine), a member of the purine alkaloids family, acts as a non-selective antagonist of A_1R and $A_{2A}R$. A_1R is widely distributed in the forebrain and hindbrain, while $A_{2A}R$ is exclusively found in the forebrain, particularly in the cerebral cortex, hippocampus, and striatum. A1R exhibits high affinity for adenosine at around 70 nM, whereas $A_{2A}R$ has lower affinity at approximately 150 nM.[24,25] Adenosine, an inhibitory neuromodulator in the CNS, exerts an anticonvulsant effect through A1R activation.[26,27] In our prior study, low-dose caffeine or 8-CPT, a selective A1R antagonist, displayed a proconvulsant effect.[9] Various animal studies have suggested that low doses of caffeine increase seizure susceptibility.[9,28] The current study's results indicated that both 10 and 50 mg/kg of caffeine significantly reduced the onset threshold for both MC twitch [Figure 1A] and THE [Figure 1B], further supporting the hypothesis that the proconvulsant effect of low-dose caffeine likely stems from A_1R antagonism.

Despite numerous reports on the proconvulsant effects of caffeine,[6,29] some doses of caffeine do not alter seizure thresholds. For instance, a dose of 20 mg/kg of caffeine had no impact on PTZ-induced seizures in adult rats.[30] Similarly, doses of 100 or 200 mg/kg did not affect the PTZ-induced seizure threshold.[31] In another study, doses of 60 or 80 mg/kg of caffeine did not influence the PTZ-

induced seizure threshold.[7] Additionally, a dose of 92.4 mg/kg of caffeine had no effect on MES in mice.[8] Consistent with our previous findings,[9] a dose of 100 mg/kg of caffeine did not significantly alter the onset threshold for MC twitch. Thus, our results align with previous studies indicating that certain doses of caffeine do not impact seizure susceptibility.

Despite having no effect on MC twitch [Figure 1A], caffeine at a dose of 100 mg/kg significantly decreased the threshold for the onset of THE [Figure 1B]. It is widely recognized that MC twitch originates from the forebrain, while THE arises from the hindbrain.[32] Caffeine exhibits a higher affinity for $A_{2A}R$ (approximately 2.4 μM) than A1R (approximately 12 nm).[33] The impact of caffeine on seizure susceptibility depends on the expression levels and types of ARs present. The differential effects of caffeine at a dose of 100 mg/kg on MC twitch and THE may be attributed to the asymmetric distribution of A1R and $A_{2A}R$ in the CNS and the dose-dependent effects of caffeine on ARs.

Given that the activation of $A_{2A}R$ increases glutamate release, the inhibitory effect of caffeine at a dose of 100 mg/kg on $A_{2A}R$ may explain why this dose does not affect MC twitch. Consistent with our findings, PTZ-induced clonic and tonic seizure thresholds were increased in $A_{2A}R$ knockout mice.[34] Our previous study using selective antagonists of A_1R and $A_{2A}R$ also suggested that caffeine at a dose of 100 mg/kg has a more inhibitory effect on $A_{2A}R$ than A_1R.[9] Additionally, $A_{2A}R$ is known to interact with A_1R, forming an A_1R-$A_{2A}R$ heterodimer in glutamatergic nerve terminals.[25] Activation of $A_{2A}R$ inhibits A_1R signaling in the heterodimer, leading to increased glutamate release.[35] It is possible that fewer A_1R-$A_{2A}R$ heterodimers are formed due to lower $A_{2A}R$ distribution in the hindbrain, potentially explaining the proconvulsant effect of caffeine at a dose of 100 mg/kg on THE through A_1R inhibition. Further experiments are needed to clarify the specific effect of caffeine at this dose on $A_{2A}R$.

NO, a neurotransmitter/neuromodulator, plays various roles in physiological and pathological functions. NO binding to its receptor, soluble guanylate cyclase (sGC), increases cGMP formation in the CNS. cGMP activates different isoforms of protein kinase G (PKG), which regulate neurotransmitter release and uptake, synaptic transmission, neuronal differentiation, and gene expression.[36] NO has been implicated in seizure susceptibility, with conflicting results in different experiments.[14-16] In our study, we utilized L-arginine (an NOS precursor), SNP (a NO donor), and L-NAME (a nonspecific NOS inhibitor) to investigate NO involvement in caffeine's effect on seizure threshold. Administration of L-arginine or SNP alone supported the proconvulsant effect of NO, with higher doses showing increased susceptibility to seizures, while L-NAME did not alter the seizure threshold [Figures 2A, 2B]. This further supports the proconvulsant role of NO in the PTZ model of seizure activity. Previous studies have shown that high doses of L-arginine can increase seizure susceptibility due to NO-induced cGMP synthesis and heightened hyper-excitability.[34]

Recent research indicates a connection between caffeine and NO. Several studies have suggested that caffeine can influence NO production. For example, the NO-cGMP pathway is involved in the enhancement of ketorolac-induced antinociception by caffeine.[18] Additionally, NO plays a role in modulating caffeine-induced locomotor activity.[19] The NO-cGMP pathway has also been linked to the anticonvulsant effect of adenosine.[12] In the current study, we observed that a dose of 100 mg/kg of caffeine significantly reduced the levels of NO metabolites in the brain. Activation of the A1R receptor decreases NO production, while activation of the $A_{2A}R$ receptor increases NO production.[17] Given the relationship between NO and adenosine, it is plausible to conclude that caffeine interacts with NO to some extent, similar to adenosine.[12,13]

In the present study, pre-treatment with normally ineffective doses of L-arginine or SNP exacerbated the proconvulsant effects of caffeine at a dose of 10 mg/kg [Figures 3A, 3B]. Conversely, pre-treatment with these same doses of L-arginine or SNP transformed the lack of effect of caffeine at a dose of 100 mg/kg into a proconvulsant effect [Figures 4A, 4B]. On the other hand, pre-treatment with normally ineffective doses of L-NAME reversed the proconvulsant effects of caffeine at both 10 mg/kg [Figures 3A, 3B] and 100 mg/kg [Figures 3A, 3B]. These findings support our hypothesis regarding the involvement of NO in the anti-seizure effects of caffeine.

Conclusions

The current findings demonstrate the dose-dependent impact of caffeine on seizure activity. Low doses of caffeine (up to 50 mg/kg) reduced the threshold for clonic and tonic seizures, whereas high doses of caffeine (100 mg/kg) specifically lowered the threshold for tonic seizures. Furthermore, through the use of L-arginine (a substrate for NOS), sodium nitroprusside (a NO donor), and L-NAME (a non-selective NOS inhibitor), we established the involvement of the NO-cGMP pathway in mediating the central effects of caffeine on seizure activity.

Acknowledgment

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Nitric oxide: NO; Nitric oxide synthase: NOS;
Adenosine receptors: Ars; Pentylenetetrazole: PTZ;
Sodium nitroprusside: SNP; Myoclonic twitch: MC;
Tonic hind limb extension: THE;
Cyclic guanosine monophosphate: cGMP;
N(G)- nitro- l- arginine methyl- ester: L- NAME;
Central nervous system: CNS; Protein kinase G: PKG;
Endothelial NOS: eNOS; Neuronal NOS: nNOS;
Inducible NOS: iNOS; Soluble guanylate cyclase: Sgc.

Authors' contributions

HJ and AH conceived and designed the experiments, conducted the experiments, and drafted the manuscript. HJ and AH analyzed the data. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval (code: IR.KaUMS.REC.1396.119) was obtained (April 2023). The present study did not interfere with the process of diagnosis and treatment of patients and all participants signed an informed consent form.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

References

1. Nieber K. The Impact of Coffee on Health. Planta Med 2017;83:1256-63. doi:10.1055/s-0043-115007 PMid:28675917
2. El Yacoubi M, Ledent C, Parmentier M, Bertorelli R, Ongini E, Costentin J, et al. Adenosine A2A receptor antagonists are potential antidepressants: evidence based on pharmacology and A2A receptor knockout mice. Br J Pharmacol 2001;134:68-77 doi:10.1038/sj.bjp.0704240 PMid:11522598 PMCid:PMC1572930
3. Tchekalarova J, Kubova H, Mares P. Postnatal caffeine treatment affects differently two pentylenetetrazol seizure models in rats. Seizure 2009;18:463-9. doi:10.1016/j.seizure.2009.04.002 PMid:19493686
4. Fredholm BB, Yang J, Wang Y. Low, but not high, dose caffeine is a readily available probe for adenosine actions. Mol Aspects Med 2017;55:20-5. doi:10.1016/j.mam.2016.11.011 PMid:27915051
5. Chroscinska-Krawczyk M, Jargiello-Baszak M, Walek M, Tylus B, Czuczwar SJ. Caffeine and the anticonvulsant potency of antiepileptic drugs: experimental and clinical data. Pharmacol Rep 2011;63:12-8. doi:10.1016/S1734-1140(11)70394-2 PMid:21441607
6. van Koert RR, Bauer PR, Schuitema I, Sander JW, Visser GH. Caffeine and seizures: A systematic review and quantitative analysis. Epilepsy Behav 2018;80:37-47 doi:10.1016/j.yebeh.2017.11.003 PMid:29414557
7. Bankstahl M, Bankstahl JP, Bloms-Funke P, Loscher W. Striking differences in proconvulsant-induced alterations of seizure threshold in two rat models. Neurotoxicology 2012;33:127-37. doi:10.1016/j.neuro.2011.12.011 PMid:22209701
8. Jargiello-Baszak M, Chroscinska-Krawczyk M, Andres-Mach M, Luszczki JJ, Czuczwar SJ. Influence of caffeine on the protective activity of gabapentin and topiramate in a mouse model of generalized tonic-clonic seizures. Pharmacol Rep 2016;68:680-5. doi:10.1016/j.pharep.2016.03.011 PMid:27116699
9. Esmaili Z, Heydari A. Effect of acute caffeine administration on PTZ-induced seizure threshold in mice: Involvement of adenosine receptors and NO-cGMP signaling pathway. Epilepsy Res 2019;149:1-8. doi:10.1016/j.eplepsyres.2018.10.013 PMid:30391360
10. Nakken KO, Solaas MH, Kjeldsen MJ, Friis ML, Pellock JM, Corey LA. Which seizure-precipitating factors do patients with epilepsy most frequently report? Epilepsy Behav 2005;6:85-9. doi:10.1016/j.yebeh.2004.11.003 PMid:15652738
11. Dworetzky BA, Bromfield EB, Townsend MK, Kang JH. A prospective study of smoking, caffeine, and alcohol as risk factors for seizures or epilepsy in young adult women: data from the Nurses' Health Study II. Epilepsia 2010;51:198-205 doi:10.1111/j.1528-1167.2009.02268.x PMid:19694796 PMCid:PMC3090289
12. Akula KK, Dhir A, Kulkarni SK. Nitric oxide signaling pathway in the anti-convulsant effect of adenosine against pentylenetetrazol-induced seizure threshold in mice. Eur J Pharmacol 2008;587:129-34. doi:10.1016/j.ejphar.2008.03.038 PMid:18457833
13. Kaster MP, Rosa AO, Santos ARS, Rodrigues ALS. Involvement of nitric oxide-cGMP pathway in the antidepressant-like effects of adenosine in the forced swimming test. Int J Neuropsychopharmacol 2005;8:601-6. doi:10.1017/S1461145705005316 PMid:16202183
14. Royes LFF, Fighera MR, Furian AF, Oliveira MS, Fiorenza NG,

de Carvalho Myskiw J, et al. Involvement of NO in the convulsive behavior and oxidative damage induced by the intrastriatal injection of methylmalonate. Neurosci Lett 2005;376:116-20. doi:10.1016/j.neulet.2004.11.038 PMid:15698932

15. Riazi K, Roshanpour M, Rafiei-Tabatabaei N, Homayoun H, Ebrahimi F, Dehpour AR. The proconvulsant effect of sildenafil in mice: role of nitric oxide-cGMP pathway. Br J Pharmacol 2006;147:935-43. doi:10.1038/sj.bjp.0706680 PMid:16474413 PMCid:PMC1760711
16. Yahyavi-Firouz-Abadi N, Tahsili-Fahadan P, Riazi K, Ghahremani MH, Dehpour AR. Involvement of nitric oxide pathway in the acute anticonvulsant effect of melatonin in mice. Epilepsy Res 2006;68:103-13. doi:10.1016/j.eplepsyres.2005.09.057 PMid:16406488
17. Bruce C, Yates DH, Thomas PS. Caffeine decreases exhaled nitric oxide. Thorax 2002;57:361-3. doi:10.1136/thorax.57.4.361 PMid:11923558 PMCid:PMC1746303
18. Lopez-Munoz FJ, Castaneda-Hernandez G, Flores-Murrieta FJ, Granados-Soto V. Effect of caffeine coadministration and of nitric oxide synthesis inhibition on the antinociceptive action of ketorolac. Eur J Pharmacol 1996;308:275-7. doi:10.1016/0014-2999(96)00320-2 PMid:8858298
19. Kayir H, Uzbay IT. Evidence for the role of nitric oxide in caffeine-induced locomotor activity in mice. Psychopharmacology (Berl) 2004;172:11-5. doi:10.1007/s00213-003-1625-5 PMid:14624327
20. Chang J, Fedinec AL, Kuntamallappanavar G, Leffler CW, Bukiya AN, Dopico AM. Endothelial Nitric Oxide Mediates Caffeine Antagonism of Alcohol-Induced Cerebral Artery Constriction. J Pharmacol Exp Ther 2016;356:106-15. doi:10.1124/jpet.115.229054 PMid:26555891 PMCid:PMC4702074
21. Heydari A, Davoudi S. The effect of sertraline and 8-OH-DPAT on the PTZ_induced seizure threshold: Role of the nitrergic system. Seizure 2017;45:119-24 doi:10.1016/j.seizure.2016.12.005 PMid:28012414
22. Mesdaghinia A, Yazdanpanah H, Seddighi M, Banafshe HR, Heydari A. Effect of short-term lead exposure on PTZ-induced seizure threshold in mice. Toxicol Lett 2010;199:6-9. doi:10.1016/j.toxlet.2010.07.012 PMid:20673792
23. El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois J-M. Evidence for the involvement of the adenosine A(2A) receptor in the lowered susceptibility to pentylenetetrazol-induced seizures produced in mice by long-term treatment with caffeine. Neuropharmacology 2008;55:35-40 doi:10.1016/j.neuropharm.2008.04.007 PMid:18486156
24. Dunwiddie T V, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001;24:31-55. doi:10.1146/annurev.neuro.24.1.31 PMid:11283304
25. Ciruela F, Casado V, Rodrigues RJ, Lujan R, Burgueno J, Canals M, et al. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 2006;26:2080-7. doi:10.1523/JNEUROSCI.3574-05.2006 PMid:16481441 PMCid:PMC6674939
26. Tescarollo FC, Rombo DM, DeLiberto LK, Fedele DE, Alharfoush E, Tomé ÂR, Cunha RA, Sebastião AM, Boison D. Role of Adenosine in Epilepsy and Seizures. J Caffeine Adenosine Res. 2020;10(2):45-60. doi:10.1089/caff.2019.0022 PMid:32566903 PMCid:PMC7301316
27. Kochanek PM, Vagni VA, Janesko KL, Washington CB, Crumrine PK, Garman RH, et al. Adenosine A1 receptor knockout mice develop lethal status epilepticus after experimental traumatic brain injury. J Cereb Blood Flow Metab 2006;26:565-75. doi:10.1038/sj.jcbfm.9600218 PMid:16121125
28. Loscher W. Preclinical assessment of proconvulsant drug activity and its relevance for predicting adverse events in humans. Eur J Pharmacol 2009;610:1-11 doi:10.1016/j.ejphar.2009.03.025 PMid:19292981
29. Luszczki JJ, Zuchora M, Sawicka KM, Kozinska J, Czuczwar SJ. Acute exposure to caffeine decreases the anticonvulsant action of ethosuximide, but not that of clonazepam, phenobarbital and valproate against pentetrazole-induced seizures in mice. Pharmacol Rep 2006;58:652-9.
30. Himmel HM. Safety pharmacology assessment of central nervous system function in juvenile and adult rats: effects of pharmacological reference compounds. J Pharmacol Toxicol Methods 2008;58:129-46. doi:10.1016/j.vascn.2008.06.001 PMid:18585470
31. Akula KK, Dhir A, Kulkarni SK. Rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor increases pentylenetetrazol seizure threshold in mice: possible involvement of adenosinergic mechanism. Epilepsy Res 2008;78:60-70. doi:10.1016/j.eplepsyres.2007.10.008 PMid:18054463
32. Wang Y-J, Hsieh C-P, Chan M-H, Chan T-Y, Chen L, Chen H-H. Distinct effects of resveratrol on seizures and hyperexcitability induced by NMDA and 4-aminopyridine. Nutr Neurosci 2019; 22:867-76. doi:10.1080/1028415X.2018.1461458 PMid:29642764
33. Florén Lind S, Stam F, Zelleroth S, Meurling E, Frick A, Grönbladh A. Acute caffeine differently affects risk-taking and the expression of BDNF and of adenosine and opioid receptors in rats with high or low anxiety-like behavior. Pharmacol Biochem Behav. 2023;227-228:173573. doi:10.1016/j.pbb.2023.173573 PMid:37302662
34. El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois J-M. Adenosine A2A receptor deficient mice are partially resistant to limbic seizures. Naunyn Schmiedebergs Arch Pharmacol 2009;380:223-32. doi:10.1007/s00210-009-0426-8 PMid:19488739
35. Ferré S, Sarasola LI, Quiroz C, Ciruela F. Presynaptic adenosine receptor heteromers as key modulators of glutamatergic and dopaminergic neurotransmission in the striatum. Neuropharmacology. 2023;223:109329. doi:10.1016/j.neuropharm.2022.109329 PMid:36375695
36. Nelissen E, Schepers M, Ponsaerts L, Foulquier S, Bronckaers A, Vanmierlo T, Sandner P, Prickaerts J. Soluble guanylyl cyclase: A novel target for the treatment of vascular cognitive impairment? Pharmacol Res. 2023;197:106970. doi:10.1016/j.phrs.2023.106970 PMid:37884069

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